Pathophysiology of coronary circulation[1]

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I consider it a great honour to have been asked to give the Sir Thomas Lewis Lecture, and I have chosen for my subject the pathophysiology of the coronary circulation – a topic which was of great interest to Sir Thomas. Another reason for selecting this topic is that so much of the good, recent work in this field has been done in Britain and many of the investigators who have made significant contributions are members of this distinguished society. This may possibly be a disadvantage in that I will have difficulty saying anything which is not well known to a large segment of the audience. A third reason for this selection is that ischaemic heart disease is of interest to me and has been the subject of much of the research with which I have been associated during the past nine years. Indeed, the Professorship which I hold at The Johns Hopkins University School of Medicine is supported by the Clayton Fund established by the late Mr. William L. Clayton and his wife to further research in arteriosclerosis, especially of the coronary arteries.

Sir Thomas Lewis has been known to me through his work throughout my professional life and I have had contact with many of his pupils. During the course of preparing this lecture I learned that Sir Thomas had come to Baltimore in October of 1914 and delivered the Herter Lectures at The Johns Hopkins Hospital and I was delighted by what I found in his introductory remarks (Lewis, 1915). His words of 55 years ago bear repeating in that they outline the philosophy upon which clinical investigation has moved forward. These remarks indicate to me that Sir Thomas would have approved of much of the active investigation of coronary artery disease and myocardial infarction, which is currently in progress. With your permission I would

like to quote briefly from the introduction to the first Herter Lecture as follows:

'Laboratory methods as applied to the study of clinical medicine have come to stay; instruments and methods of precision are gradually relieving medicine of its past stigma; they are lifting it to the plane of its sister sciences, its true and proper status. We have been too content in the past with opinion. In the future we shall rest our case upon fact.' (Lewis, 1915.)

I would like to turn to the subject of my lecture by examining the history of clinical investigation of ischaemic heart disease. The modern era begins with Sir Thomas who in his Billings Lecture in Chicago in 1931 summarized the descriptive observations which had gone before and pointed the way for future studies (Lewis, 1932). The first major investigative study on this subject came from his department, the Department of Clinical Research at the University College Hospital. I refer to the work of Wayne and Laplace published in *Clinical Science* in 1933. This represents an important milestone along the road to the understanding of the pathophysiology of angina pectoris. This is a classic of clinical investigation, and I point to it as an example of what can be accomplished by simple well-designed experiments and thoughtful interpretation. This was budgetless research. Pulse and blood pressure were measured during 400 attacks of chest pain induced by exercise. Observations of great interest abound in this paper. Of special interest is the observation of the effect of atropine on exercise tolerance and the onset of pain as depicted in Fig. 1.

The investigators measured blood pressure systolic and diastolic, pulse rate, and the onset and cessation of pain. The number of efforts of ascents over a step are recorded as the height of the vertical bar. The panel at the left displays the results of the control experiment and the comparable observations after atropine are seen in the right-hand panel. It is readily apparent that the control pulse rate is

higher and the post exercise tachycardia of longer duration after atropine. The patient was able to do only approximately half as much work after atropine and the pain persisted for a longer time. It is of interest that the blood pressure after atropine was lower than in the control state. These experiments provide a clear demonstration of the importance of pulse rate in the precipitation of angina. This effect has been demonstrated many times since 1933 but never with more clarity. The design was simple, the results clear, and hence interpretation was easy.

Wayne concluded from the atropine experiments that, 'The diminution in the number of efforts (trips up the steps) and the increase in the duration of the pain under atropine . . . can be attributed . . . to the more rapid action of the heart, which increases its energy expenditure without a concomitant increase in coronary flow. . . . .' These atropine experiments can be considered to be anticipatory of the studies with pacing-induced tachycardia which have occupied many of us in the past few years.

It is also of note that in this same paper Wayne and Laplace reported observations on the effect of mechanical stimulation of the carotid sinus on angina pectoris. Thus, another area of interest in the seventh decade of the century was pioneered in Sir Thomas Lewis' laboratory in the fourth decade.

Haemodynamics of myocardial ischaemia

In the late 1950's the technique of right heart catheterization was applied to the study of angina by Müller and Rørvik (1958) in Norway and by Johnson, Fairley, and Carter (1959) in the United States. Both groups emphasized the many similarities between angina and dyspnoea as manifestations of congestive failure. Both groups recorded increased pulmonary artery and pulmonary capillary wedge pressures in association with anginal pain. After these two significant papers, the slow pace of clinical investigation of ischaemic heart disease continued until the latter half of the current decade. Between 1965 and the present time, many significant papers have been published on this subject; this current world-wide epidemic of publication being attributed to the introduction of three new techniques. The first is the development of coronary arteriography, the second is revascularization surgery, and the third is the development of techniques whereby the heart rate can be artificially controlled. Medical historians may well look back on this
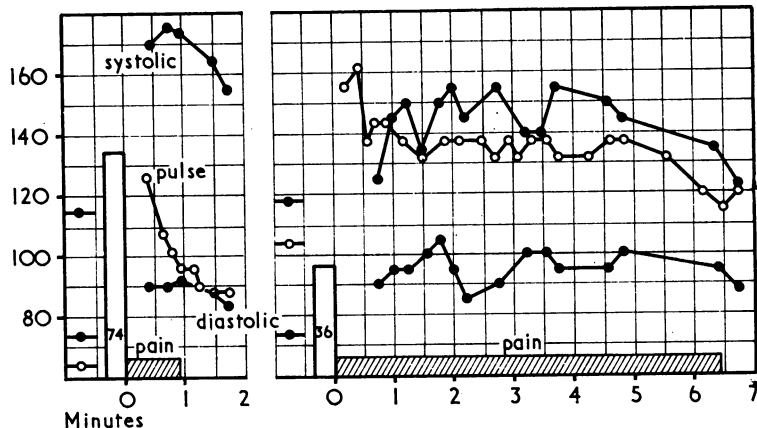

FIG. 1   *The effect of atropine-induced tachycardia on exercise tolerance. Observations after atropine are shown in the right-hand panel and compared to control observation on the left. The number in the clear vertical bar is the number of ascents over a step and the length of the horizontal hatched bar represents the duration of pain. Reproduced from Wayne and Laplace (1933) by permission of* Clinical Science.

current decade and list coronary arteriography as one of the important developments, but may well consider it to be important because it brought many patients with ischaemic heart disease into the haemodynamic laboratory for study. Before 1960, few patients with coronary heart disease were subjected to haemodynamic evaluation, as such a study would have been purely investigational and without any potential for therapeutic benefit.

The active investigation of the past five years has contributed to the current concept of the pathophysiology of myocardial ischaemia depicted in Fig. 2 in the form made popular by the systems analyst. The central point of this diagram is the equality between oxygen supply and demand at the cellular level. On the left are the mechanical factors that influence demand, heart rate, wall tension, and myocardial contractility. On the right are the several factors that influence supply, all of which are related to blood flow. At the bottom are listed the manifestations of ischaemia which are the consequences of an imbalance between supply and demand.

This diagram could be rendered more comprehensive and hence more complicated by the addition of all the feedback relationships which can be postulated. For example, vasoactive substances may reduce coronary vascular resistance and hence tend to correct the situation that produced the ischaemia. On
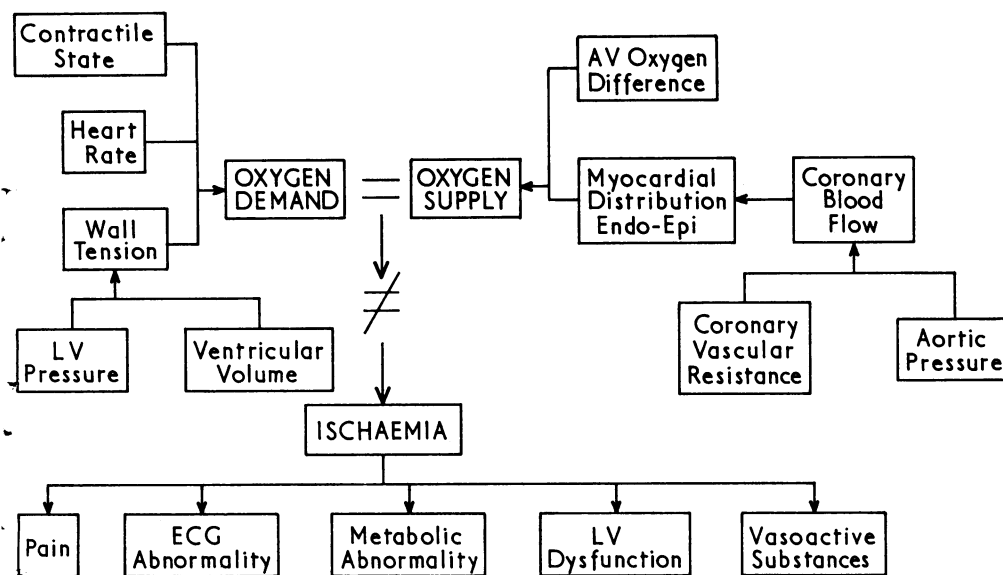
the other hand, deterioration of left ventricular function results in an increase in ventricular volume and wall tension, which leads to increased oxygen demand and further ischaemia. Alterations in left ventricular function and wall tension may also have deleterious consequences on myocardial blood flow distribution.

A lecture could be devoted to many aspects of this diagram but I have elected to confine my attention to only three aspects with which the group of investigators in my laboratory have been concerned. First, we will discuss Dr. G. C. Friesinger's work on the relation between ischaemia and left ventricular dysfunction followed by the work of Dr. B. Pitt and Dr. C. R. Conti with myocardial blood flow in association with angina and the possible role of vasoactive substances in its control, and then, the studies of Drs. Pitt, N. J. Fortuin, and L. Becker on the myocardial microcirculation as studied with microspheres.

First, in accordance with this plan let us discuss the consequences of ischaemia on left ventricular function. In 1962, during the course of arteriographic study we made observations of left ventricular pressure during an anginal attack (Ross et al., 1962). We define anginal pain as substernal pain or discomfort of major severity, which is described as a squeezing constriction or pressure which is identical to the sensation the patient

experiences on exertion. In Fig. 3 the left ventricular end-diastolic pressure is seen to rise before the onset of chest pain. The pressure rise is associated with the appearance of a large 'a' wave. The chest pain disappeared after the administration of nitroglycerin and the left ventricular pressure returned to normal. These direct observations of left ventricular pressure confirmed the indirect observations of Müller and Rørvik (1958), and Johnson et al. (1959) who recorded increases in pressures in the pulmon-
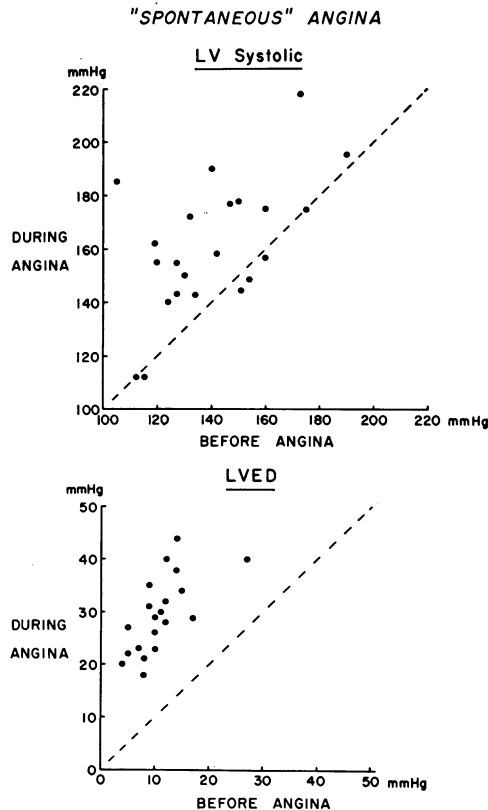
FIG. 3 *Left ventricular diastolic pressure before, during, and after a spontaneous attack of angina pectoris. Note the appearance of a large 'a' wave in the record of 3.04 pm. Anginal pain appeared one minute later at 3.05 pm and responded promptly to nitroglycerin.*
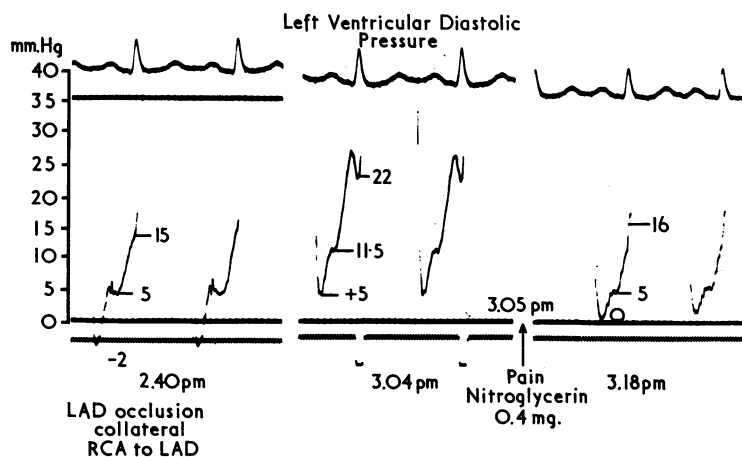
ary circulation during angina. The direct observations clearly indicated that the primary alteration in physiology was in the left ventricle and not in the pulmonary circulation. Fortunately for the patients and unfortunately for the investigator, angina pectoris occurs relatively rarely during coronary arteriography.

Dr. Friesinger has collected observations on 20 patients who have developed spontaneous attacks of angina pectoris during cardiac catheterization, and in Fig. 4 the results of these studies are summarized (G. C. Friesinger, unpublished observations). The left ventricular end-diastolic pressure is consistently raised in association with anginal pain. The heart rate did not change significantly, there being an increase of more than 10 beats a minute in only 3 patients. The systolic blood pressure was raised in some but not in all

patients. No relation was apparent between the magnitude of the blood pressure rise and the rise in left ventricular end-diastolic pressure. We have carefully examined the possibility that these haemodynamic alterations might be attributed at least in part to the administration of contrast material and feel certain that this is not the case. As further evidence for this, we have examined the haemodynamic changes that follow injections of contrast material which are not associated with anginal attacks. In only 2 of 23 such patients did the left ventricular end-diastolic pressure exceed 20 mm. Hg.

The two possible explanations for the observation of increased left ventricular end-diastolic pressure in association with angina pectoris are presented diagrammatically in Fig. 5. This figure is based upon the pressure volume loop of the left ventricle shown at the left of the figure. In this presentation, left ventricular pressure is plotted on the ordinate and volume on the abscissa. At the lower left-hand corner of the loop, the mitral valve opens and the diastolic filling of the ventricle begins and continues along the portion of the loop which parallels the volume axis (V). At the right-hand corner, the left ventricle pressure rises and the mitral valve closes. At the upper right, the aortic valve opens and ejection begins, and at the upper left, ejection is terminated and the aortic valve closes.

To illustrate the two explanations for the increased end-diastolic pressure in ischaemic heart disease, the bottom of this loop or the

FIG. 4 *Haemodynamic consequences of spontaneous angina pectoris. LV systolic, peak left ventricular systolic pressure (top panel). LVED, left ventricular end-diastolic pressure (lower panel). Observations before the onset of angina are plotted on the abscissa and those obtained during the anginal attack are plotted on the ordinate.*

FIG. 5 *Pressure volume loop of the left ventricle. Theoretical considerations of alterations which may be associated with spontaneous angina.*
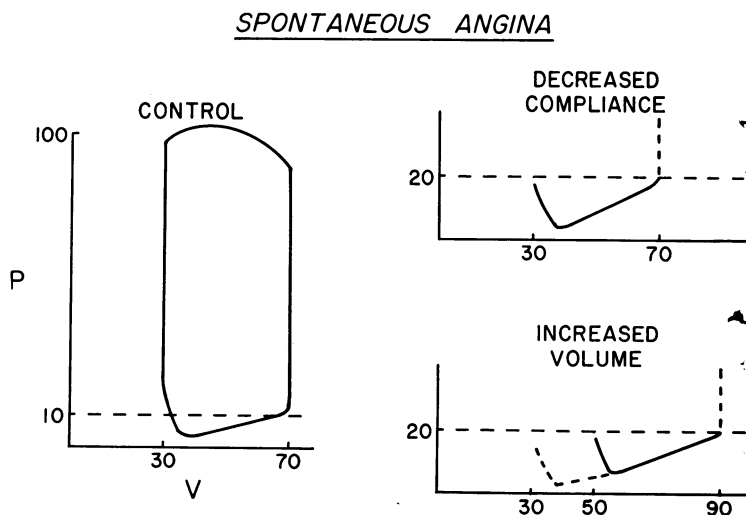
diastolic filling phase has been enlarged and modified in the two panels at the right. At the top, the effect of decreased compliance of the ventricle with no change in volume is shown. The left ventricular end-diastolic pressure is increased because the pressure volume curve rises more steeply with decreased compliance, and hence a higher pressure is reached with the same filling volume. In the lower diagram the compliance has remained unchanged but the rise in end-diastolic pressure has been brought about by an increase in volume. Therefore, the pressure change that has been observed could have resulted from either an increase in volume or a decrease in compliance, but the evidence to be presented suggests that decreased compliance is a more likely explanation.

The introduction by Sowton *et al.* (1967) of the technique of atrial pacing for the study of angina pectoris represented a major step forward in this investigation and helped provide an answer to the question posed by the observation on spontaneous angina. This story would have given Thomas Lewis pleasure as it illustrates so beautifully the close interaction between the clinic and the laboratory. Physiologists have for many years used repetitive electrical stimulation to control heart rate of experimental animals, but only recently has the technique been brought to the clinic for use in the management of heart block. Now the clinical technique is taken back into the laboratory and used as a powerful tool for clinical investigation of angina pectoris.

Pacing provides a safe reproducible method whereby the oxygen demands of the myocardium can be increased while the patient is at rest on the cardiac catheterization table. The changes in peripheral circulation which accompany exercise are not present. Catheters can remain in the left ventricle, coronary arteries, and coronary sinus during pacing, and hence measurement can be made which would not be possible during exercise.

The records of a typical pacing study performed by Dr. Friesinger are shown in Fig. 6, 7, and 8. In Fig. 6, the resting heart rate is 72 and the left ventricular pressure is easily measured at the termination of the 'a' wave. A pacing catheter in the right atrium or coronary sinus is used to increase the heart rate by 10 beats a minute increments until anginal pain or ST segment changes appear. In Fig. 7 the paced heart rate is 110 beats a minute and it is not possible to clearly identify the point at which the end-diastolic pressure should be measured. It can be seen, however, that the early and lowest diastolic pressure
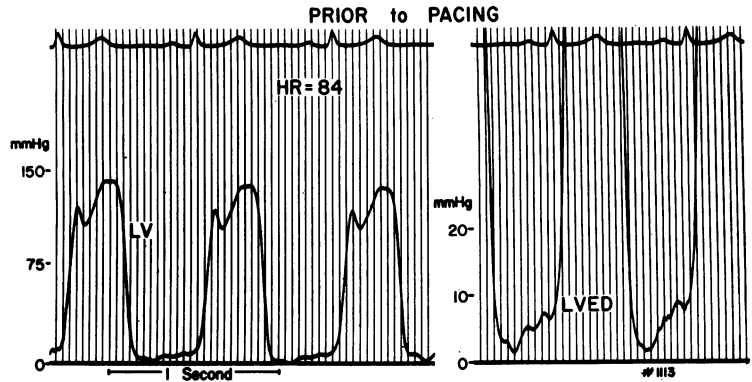

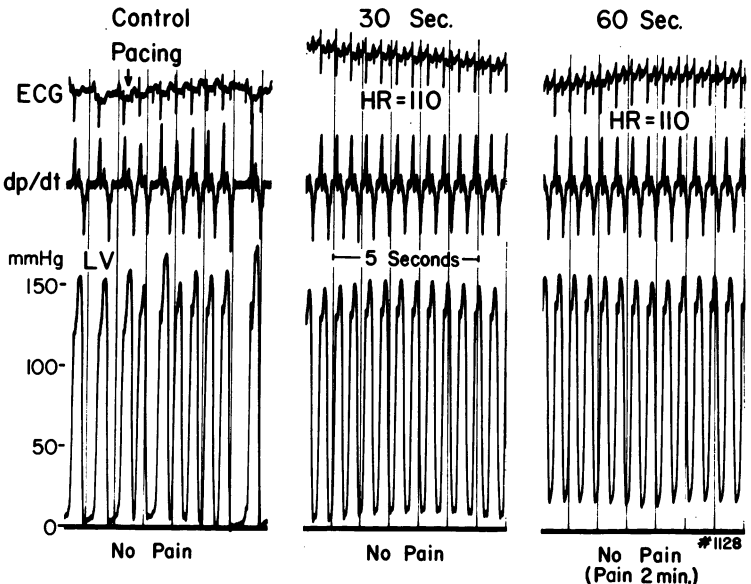FIG. 6   *Left ventricular pressure before the beginning of pacing.*

FIG. 7   *The onset of pacing (left panel). The middle and right-hand panels show recordings obtained at 30 and 60 seconds after the onset of pacing. Left ventricular diastolic pressure has risen but one patient remains symptomatic.*

has risen and that this rise preceded the onset of pain. In Fig. 8, the pacemaker has been turned off and the left ventricular end-diastolic pressure, which is difficult to measure during pacing, is clearly raised in the beats which immediately follow the end of the pacing. The pressure can be seen to rise during the first two beats at the slower spontaneous rate.

In Fig. 9, the experience with 31 patients subjected to pacing stress testing has been summarized (Friesinger, Conti, and Pitt, 1967). In the bottom panels it can be noted that in the absence of angina there was no

FIG. 8 *Pacing is terminated after the appearance of pain.*

FIG. 9 *Experience with 31 patients subjected to pacing stress testing. Observations on patients who developed angina in response to pacing are shown in the two right-hand panels and observations on those who did not develop angina are shown at the left. Left ventricular (LV) systolic pressure is shown in the top panels and left ventricular diastolic pressure (LVED) in the lower panels. In all four panels observations during pacing are plotted on the ordinate against control observations on the abcissa. Patients with arteriographically normal coronary arteries are represented by (X) and patients with ischaemic heart disease (IHD) by solid dots (●).*
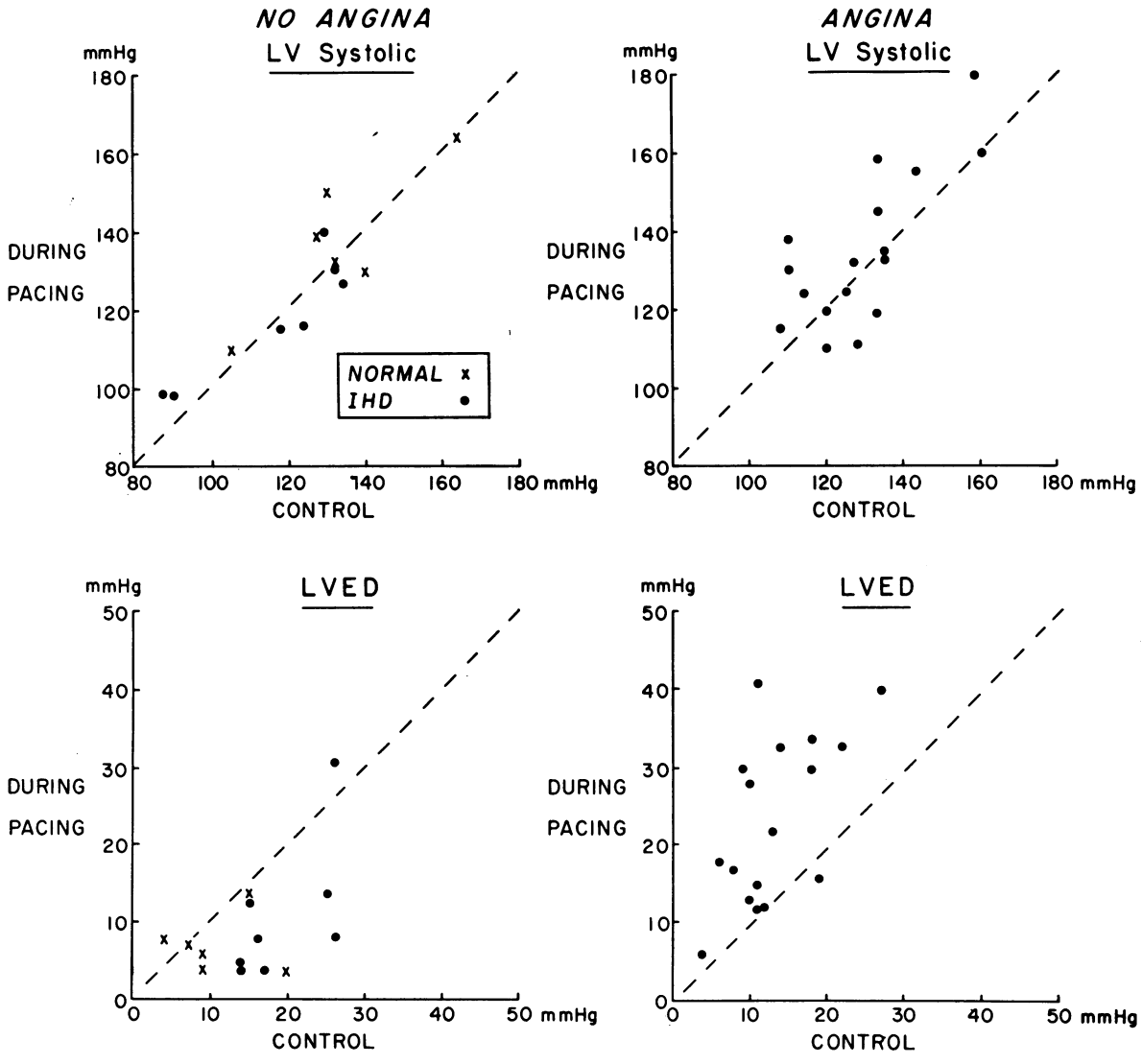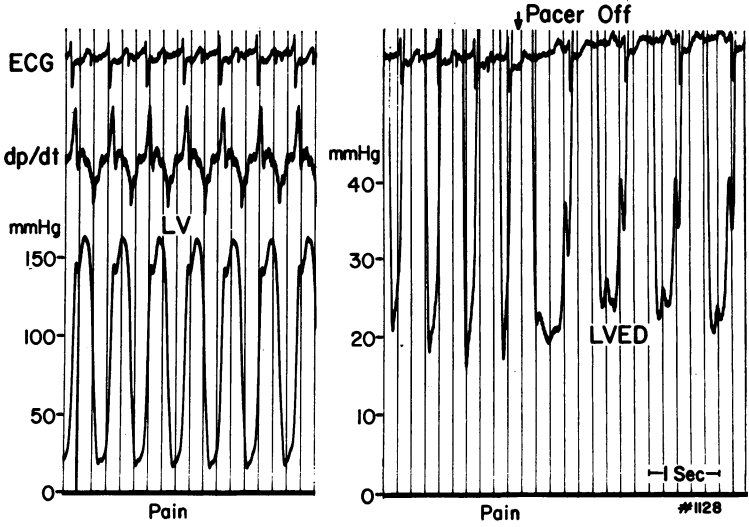
rise in left ventricular end-diastolic pressure. There was an increase in pressure in 13 of 17 patients who developed angina and 2 of the 4 patients who did not exhibit a rise had chest pain which was considered to be atypical angina pectoris. There was no consistent relation between the rise in systolic blood pressure and the increase in left ventricular end-diastolic pressure. The failure of other investigators to confirm this relation between haemodynamic changes and anginal pain may be attributed to difference in the definition of angina and hence variation in the duration of the pacing stress. Another explanation may be found in the difficulty encountered in measuring the left ventricular end-diastolic pressure during pacing at rapid rates. When this difficulty has been encountered we have measured the end-diastolic pressure on the beat following the termination of pacing.

A concept of the haemodynamic consequences of myocardial ischaemia occurring in a variety of circumstances is summarized in Fig. 10. The three factors which relate to the haemodynamics of the left ventricle are shown across the top of the table. End-diastolic pressure has been measured; volume cannot be measured directly but it can be predicted from other observations, and compliance, as discussed in conjunction with Fig. 5, is the reciprocal of stiffness. The higher the pressure rise per unit of volume, the less the compliance. It has been pointed out that the increase in left ventricular end-diastolic pressure could result either from an increased filling volume or a decreased compliance.

The observations made with the pacing technique enable us to differentiate between these two possibilities. More specifically, we know that ventricular volume is decreased during pacing, as indicated by Sowton *et al.*'s ingenious radiographic studies (1967). Additional evidence for a decreased volume during pacing is provided by the work of John Parker

of Kingston, Ontario, and others who clearly showed that stroke volume decreases as the heart rate is accelerated while the cardiac output remains constant (Parker *et al.*, 1969). Thus, the left ventricular end-diastolic pressure is increased during pacing-induced angina and ventricular volume is decreased, and, therefore, the compliance of the ventricle must be decreased. After pacing, the stroke volume returns to normal, the decreased compliance persists, and, therefore, the increase in end-diastolic pressure is augmented.

At this point in the development of the argument it is reasonable to ask whether pacing-induced angina is the same as that which occurs spontaneously. As a partial answer to this question we can show the record of one of three patients who developed angina pectoris both with pacing and spontaneously. In Fig. 11 are displayed the heart rate, systolic blood pressure, and left ventricular end-diastolic pressure measurements obtained during two spontaneous attacks of angina pectoris and during two attacks produced by pacing. First, let us consider the two spontaneous attacks and note that systolic blood pressure was 140 mm. Hg in one, and 120 mm. Hg in the other. Heart rate was the same and left ventricular end-diastolic pressure was raised in both. During the first period of paced tachycardia, the heart rate was increased to 170 beats a minute without the onset of pain and with a decrease in left ventricular end-diastolic pressure. Subse-

FIG. 11 *Haemodynamic observations in single patients during five attacks of angina. A, angina; TNG, nitroglycerin; LVED, left ventricular end-diastolic pressure.*
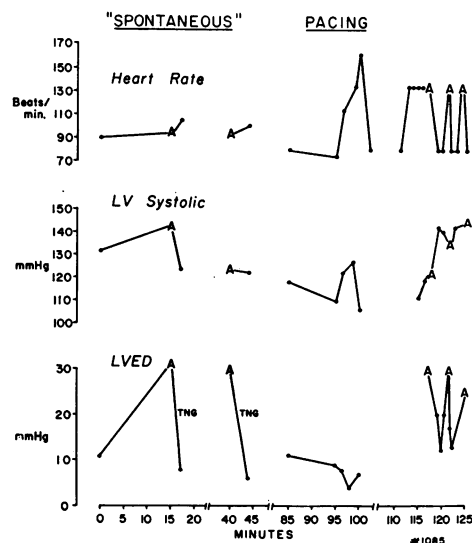

FIG. 10 *Possible explanations for the haemodynamic consequences of myocardial ischaemia.*

| ANGINA | LEFT VENTRICULAR | | |
	End Diastolic Pressure	Volume	Diastolic Compliance $\Delta V/\Delta P$
SPONTANEOUS	↑	↑	O
		O	↓
PACING	↑	↓	↓
POST PACING	↑	O	↓

quently, lesser degrees of pacemaker-induced tachycardia were associated with both anginal pain and a rise in left ventricular end-diastolic pressure. The heart rates required to produce angina with pacing are lower than those associated with the spontaneous attacks, an observation reflecting the lesser ventricular volume and hence wall tension during pacing. The conclusion from these studies is that increased left ventricular end-diastolic pressure is a consistent accompaniment of anginal pain.

Myocardial blood flow during ischaemia

I would like now to turn from haemodynamics to blood flow and report some recent observations made by Dr. Conti and Dr. Pitt on myocardial blood flow during pacing. These studies were stimulated by the observations made by Dr. Pitt in association with Dr. Donald Gregg in dogs with surgically induced heart block and implanted electromagnetic flowmeters (Pitt and Gregg, 1968). It was shown that as heart rate was increased by pacing, the cardiac output rose initially, but soon reached a plateau, and failed to increase more with further increases in rate. In contrast, it is seen that coronary blood flow continues to rise as the heart rate is increased by the pacemaker. This progressive rise in coronary flow which is linked to heart rate reflects the increase in myocardial oxygen consumption which is associated with tachycardia. These observations suggested that a similar technique might be applied in clinical investigation to show the inability of patients with ischaemic heart disease to increase myocardial blood flow in proportion to the increased myocardial oxygen consumption required by the tachycardia.

We used the xenon method to study the response of myocardial blood flow to pacing-induced tachycardia in patients (Ross *et al.*, 1964; Conti *et al.*, 1970). A saline solution of radioactive xenon is injected through the arteriographic catheter into the coronary artery, and after injection the disappearance of radioactivity from the heart is monitored by a scintillation counter positioned over the praecordium. The rate of disappearance of radioactivity is proportional to the blood flow to the region into which the gas has diffused. The larger the flow, the steeper the disappearance curve.

The results of a single experiment are shown in Fig. 12 to illustrate the experimental procedure. Myocardial blood flow has been measured by xenon disappearance before, during, and after pacing-induced tachycardia.
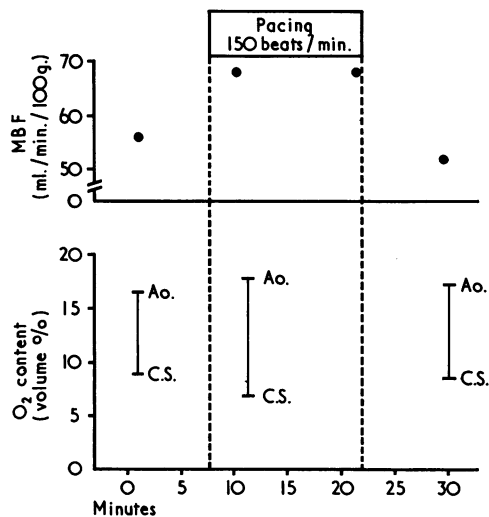
FIG. 12   *Myocardial blood flow changes in association with pacing-induced tachycardia. Ao, aorta; CS, coronary sinus; MBF, myocardial blood flow.*

Arterial and coronary sinus oxygen content have also been measured. This patient did not develop ischaemia as manifest by chest pain or electrocardiographic change during the period of tachycardia and the expected rise in myocardial blood flow occurred. It was anticipated that a patient who developed ischaemia would have a rise in myocardial blood flow of less magnitude.

The data from 28 patient studies similar to that shown in Fig. 12 are summarized in Fig. 13 (Conti *et al.*, 1970). Fourteen of the 28 patients developed ischaemia during pacing as manifest by chest pain or electrocardiographic change. Prepacing values are plotted on the horizontal axis and values obtained during pacing are plotted vertically. The solid symbols represent patients who exhibited an ischaemic response, and the open symbols those who did not. The results were not those that were anticipated because a larger increase in myocardial blood flow was recorded in the patients with an ischaemic response. The average increase in the nonischaemic group was 12 ml./min. per 100 g. while that in the ischaemic group was 25 ml./min. per 100 g. The difference between the two groups was statistically significant at the 5 per cent level. This response was the opposite of that which was anticipated and a number of explanations for this set of observations can be offered.

One possible explanation is that the area of ischaemic myocardium is relatively small compared to the entire mass of myocardium in which flow is measured by the xenon wash-
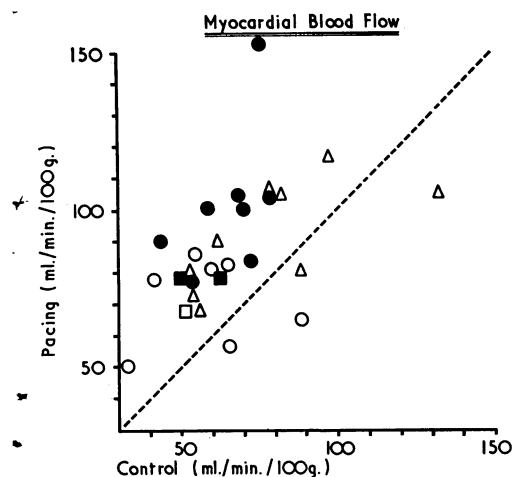
Myocardial Blood Flow

FIG. 13 *Myocardial blood flow response to pacing-induced tachycardia in 28 patients. Solid symbols identify patients with an ischaemic response to pacing as manifested by chest pain or electrocardiographic change. Open symbols identify patients who had neither manifestation of ischaemia.*

out. The flow in the normal, nonischaemic area is increased because it has to do more work to compensate for the dysfunction of the ischaemic area and hence its oxygen consumption and flow are increased (Rees and Redding, 1969). An alternative or perhaps an additional explanation would be the liberation of a substance from the ischaemic tissue which produced dilatation in the surrounding areas. The same substance might produce pain and hyperaemia. This might well be the 'P' substance with which Thomas Lewis was concerned (1932). More recent work with vasoactive substances in ischaemia has led to the suggestion by Berne that adenosine may be liberated from ischaemic tissue and serve as a powerful vasodilator (Rubio and Berne, 1969). Another powerful vasoactive substance is bradykinin which has been shown to be a coronary vasodilator and, under other circumstances, to produce pain (Maxwell, Elliott, and Kneebone, 1962; Armstrong et al., 1954). These two facts suggested that bradykinin might play a role in the haemodynamic response to myocardial ischaemia and, indeed, might be the substance responsible for the ischaemic pain, the search for which was initiated by Thomas Lewis.

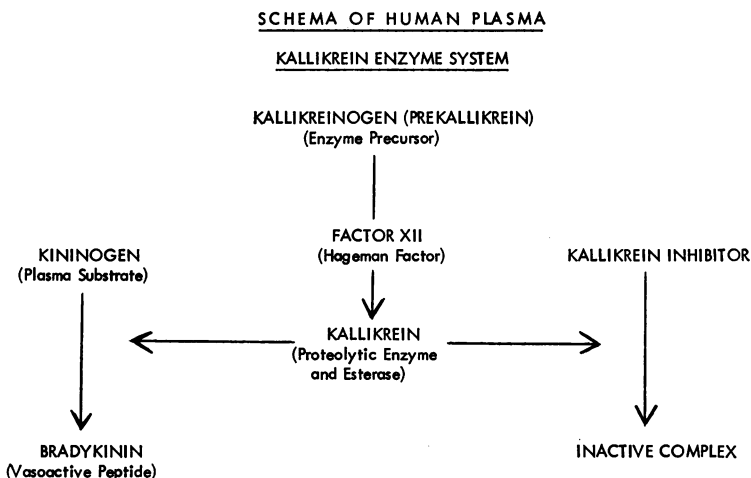## Vasoactive substances – the kallikrein system

These considerations of the possible role of the kallikrein system and bradykinin in

angina pectoris led to a series of experiments in which the kallikrein system was studied in patients during pacing-induced myocardial ischaemia. This was a collaborative effort with the blood samples being collected during pacing studies, identical to those previously described, performed at The Johns Hopkins Hospital by Drs. Pitt and Conti and the kallikrein assays being performed by Drs. Colman and Mason at the Massachusetts General Hospital (Pitt et al., 1969).

The operation of the kallikrein system of plasma is outlined in Fig. 14. Bradykinin is the pain-producing substance which is also a vasodilator and it is the end-product of a reaction which is initiated by Factor XII or the Hageman Factor. As a result of activation, the concentration of kallikreinogen in the serum falls. It is kallekreinogen which is measured and, therefore, a decrease in the concentration of this substance is indicative of activation of the system.

The results of kallikreinogen assays on samples of blood collected from the coronary sinus and aorta of 17 patients before, during, and after right atrial pacing are shown in Fig. 15. The values obtained at rest are plotted on the horizontal axis and those obtained during pacing are shown on the vertical axis. It must be remembered that a decrease in kallikreinogen indicates that the system has been activated. Patients who developed ischaemia as manifested by anginal pain or electrocardiographic change are indicated by solid figures. It is noteworthy that no patient showed activation of the system in the absence of manifestations of ischaemia. All values in

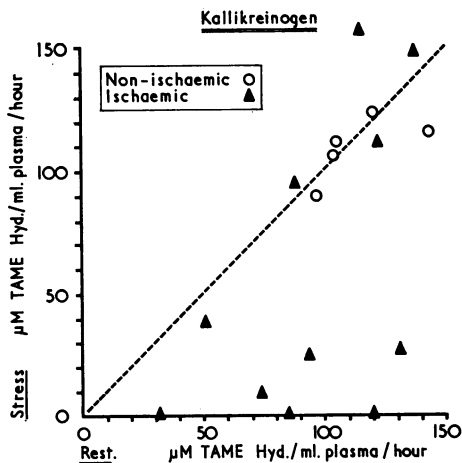FIG. 14 *Schema of human plasma kallikrein enzyme system (after Sherry). (From Pitt et al., 1969.)*

SCHEMA OF HUMAN PLASMA

KALLIKREIN ENZYME SYSTEM

KALLIKREINOGEN (PREKALLIKREIN)
(Enzyme Precursor)

FACTOR XII
(Hageman Factor)

KININOGEN
(Plasma Substrate)

KALLIKREIN INHIBITOR

KALLIKREIN
(Proteolytic Enzyme
and Esterase)

BRADYKININ
(Vasoactive Peptide)

INACTIVE COMPLEX

FIG. 15 *Activation of the kallikrein enzyme system in response to pacing-induced tachycardia.*

the nonischaemic group remained in the normal range. Eleven patients developed ischaemia with pacing, and in 7 of these the kallikrein system was activated as indicated by a fall in the kallikreinogen concentration. In 4 patients with ischaemic manifestations which were identical to those in the other 7, no activation occurred. These 4 patients without activation had pain and, therefore, it is difficult to attribute the pain to bradykinin. Myocardial blood flow was measured in only 3 of the patients with kallikrein activation and raised values were obtained in all of these. There was no significant difference between the concentrations of kallikreinogen in aorta and coronary sinus, so it is impossible to localize the activation in the heart. Though not always present in ischaemia, kallikrein activation never occurred in its absence. The significance of these findings is unknown but the role of this system in the response to ischaemia deserves further investigation.

Myocardial microcirculation

I would like to turn now to the third phase of the lecture and discuss the possible role of the microcirculation and transmyocardial distribution of blood flow in the pathophysiology of ischaemic heart disease. Thomas Lewis said, and I quote, 'It appears almost necessary to assume that the strict relation between pain and the energy expended is broken by some factor of interference and the factor in mind is an inconstant state of the coronary vessels' (Lewis, 1932).

Thomas Lewis was undoubtedly thinking of coronary spasm when he wrote these words, but I would like to suggest transmyocardial blood flow distribution in the microcirculation as another possibility. To direct your attention to this important area I would like now to present some animal experiments in which the microcirculation was studied by radioactive microspheres. There is as yet no method whereby similar observations can be made in man, but it seems likely that the distribution of blood across the myocardium is important in man both in health and in the presence of coronary atherosclerosis. Therefore, it is appropriate to include a discussion of the observations of Drs. Pitt, Fortuin, and Becker, which may possibly lead the way for future investigation (Fortuin *et al.*, 1969; Becker, Fortuin, and Pitt, 1971).

The radioactive microsphere method for the study of the myocardial circulation is outlined in Fig. 16. Radioactive microspheres 15 μm in diameter are labelled with radioactive material, and a suspension of the radioactive microspheres is injected through a catheter into the left atrium of the experimental animal. The microspheres are distributed throughout the body in accordance with the distribution of the blood flow and by virtue of their size become wedged in precapillary vessels throughout the body. The number of spheres is small relative to the number of precapillary vessels in the vascular bed, and there are no haemodynamic consequences of sphere injection. The most sensitive indicator of the capacity of the vascular bed to dilate is the magnitude of the reactive hyperaemic response and it can be shown that this

FIG. 16 *Diagrammatic representation of microsphere method for determination of regional myocardial blood flow.*
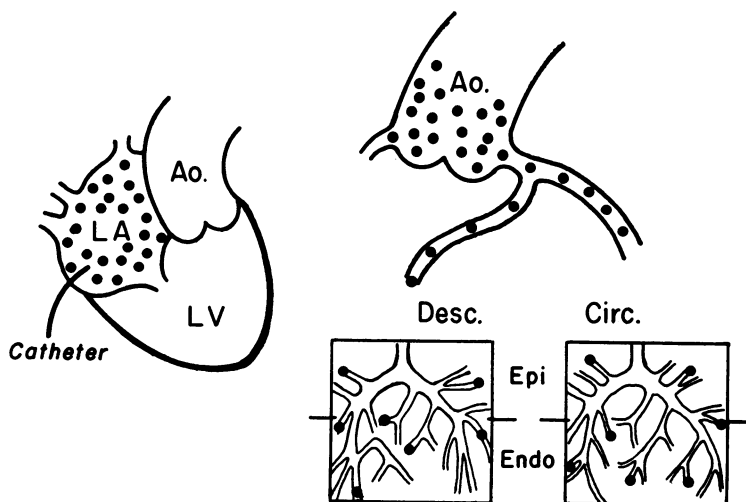
response is not blunted by repeated injections of microspheres.

At the conclusion of the experiment the animal is killed and samples of myocardium are removed, weighed, and the radioactivity determined. This radioactivity is expressed as counts per unit weight of myocardium and this value for one region of the myocardium compared to a similar value for another region. The ratio of counts per gramme from one region to a comparable value from another region reflects the ratio of blood flow of the two regions. In the example in Fig. 16, the ratio of blood flow in the area perfused by the anterior descending coronary artery to that in the area perfused by the circumflex would be 1. It is also possible to slice the myocardium parallel to the epicardial surface and measure flow in the endocardial and epicardial portions separately. In the example in Fig. 16, the ratio of flow in the endocardium to that in the epicardium would be 4:3.

An essential feature of the method is the fact that spheres can be labelled with different isotopes and the isotopes measured separately. Thus, it is possible to obtain 15 μm microspheres labelled with three different isotopes. An injection of spheres labelled with isotope No. 1 is made, and then an intervention can be carried out after which an injection of spheres labelled with isotope No. 2 will be made. If necessary, a third injection with isotope No. 3 can also be made. After death, differential counting for the three isotopes is carried out to obtain three different sets of flow measurements in relation to the experimental intervention.

The result of a typical experiment showing the distribution of microspheres, and hence blood flow, are shown in Fig. 17. It is apparent that the flow to the area perfused by the circumflex coronary artery is equal to the flow

to the area of the anterior descending (circ/desc = 0·98). There is, however, a significant difference between endocardial and epicardial blood flow in the circumflex region of the left ventricle with a significantly greater blood flow to the endocardium (circ endo/epi = 1·17).

The results of experiments designed to show the effects of ischaemia on microsphere distribution and hence blood flow are presented in Fig. 18. An injection of microspheres was made before and 5 minutes after acute occlusion of the circumflex coronary artery by a snare. Analysis revealed that there was a decrease in the circumflex descends ratio from 1·00 to 0·22 reflecting the ischaemia in the area of circumflex perfusion. Of more
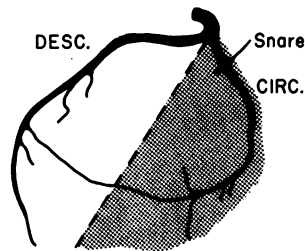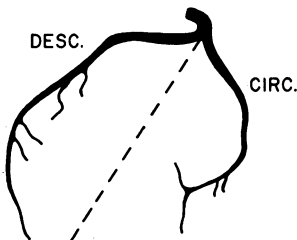
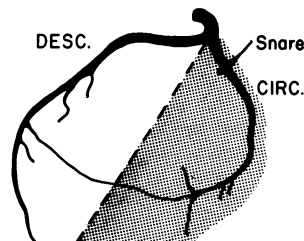|	$\frac{CIRC}{DESC}$	$DESC\frac{ENDO}{EPI}$	$CIRC\frac{ENDO}{EPI}$
CONTROL	1.00	0.99	1.13
OCCLUSION	0.19*	1.00	0.85*
OCCLUSION	0.22	1.03	0.88

*p<0.05

FIG. 18   *Effect of ischaemia on myocardial blood flow distribution in 7 anaesthetized dogs as measured by microspheres. (From B. Pitt, L. Becker, and N. J. Fortuin, to be published.)*

FIG. 19   *Effect of nitroglycerin on the myocardial blood flow distribution in ischaemia in 11 anaesthetized dogs.*

	$\frac{CIRC}{DESC}$	$DESC\frac{ENDO}{EPI}$	$CIRC\frac{ENDO}{EPI}$
CONTROL	0.98	1.06	1.21
OCCLUSION	0.19*	1.05	0.84*
OCCLUSION and NITROGLYCERIN	0.22	1.16*	1.01*

*p<0.05

FIG. 17   *Myocardial blood flow distribution in 28 anaesthetized dogs measured by microspheres.*

	$\frac{CIRC}{DESC}$	$DESC\frac{ENDO}{EPI}$	$CIRC\frac{ENDO}{EPI}$
NORMAL	0.98	1.04	1.17

interest, however, is the decrease in the endo/epi ratio in the ischaemic area indicating that the ischaemia is most severe in the endocardial portions of the myocardium. This finding that ischaemia is most severe in the endocardium is consistent with the clinical observation which indicates that the subendocardial regions are most vulnerable to ischaemia during exercise and in myocardial infarction.

The microsphere studies have also added useful information concerning the mechanism of action of nitroglycerin. The experiment depicted in Fig. 19 is similar to that in Fig. 18 but an injection of microspheres has been made after an occlusion which was preceded by the administration of nitroglycerin. Nitroglycerin did not result in an increase in the circ/desc ratio and hence, there was no increase in total flow into the area of ischaemia distal to the occlusion. There was, however, an increase in the endo/epi ratio in the ischaemic area which indicates that the blood flow to the endocardium had been improved.

Much has been learned about the pathophysiology of ischaemic heart disease since the time of Sir Thomas Lewis, and the rate at which new information is being acquired is increasing. Emphasis has shifted from haemodynamics to considerations of vasoactive substances and myocardial blood flow distribution. Many questions first posed by Sir Thomas Lewis still remained unanswered and foremost among these is the question concerning the cause of ischaemic pain.

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